The Brine Shrimp Artemia. *1980. Vol. 3. Ecology, Culturing, Use in Aquaculture*
G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

International Study on *Artemia*[1] IV. The biometrics of *Artemia* strains from different geographical origin

Paul Vanhaecke and Patrick Sorgeloos[2]

Artemia Reference Center, State University of Ghent
J. Plateaustraat 22, B-9000 Ghent, Belgium

Abstract

The cosmopolitan distribution of brine shrimp in coastal lagoons and island salt lakes has resulted in numerous geographical strains.

In the framework of the characterization study of different *Artemia* strains which is the objective of the laboratories participating into the "International Study on *Artemia*" the following biometrical parameters have been studied by the Artemia Reference Center :

- volume and diameter of hydrated, untreated and decapsulated cysts ;
- chorion thickness ;
- length, dry weight and ash free dry weight of freshly hatched nauplii ;
- volume index of freshly hatched nauplii.

For all parameters, important differences were observed between the *Artemia* strains studied. From these data it appears that many strains can be differentiated on the basis of their biometrical characteristics. The small variations observed in a few cases between batches of the same strain might be caused by fluctuating environmental conditions and/or cyst processing techniques.

Highly significant correlations have been found between a number of the biometrical parameters which were taken into consideration ; this will, no doubt, facilitate the further screening of *Artemia* strains.

The impact of the biometrical characterization of *Artemia* strains on the practical use of brine shrimp in aquaculture is discussed.

Introduction

The brine shrimp is a cosmopolitan organism inhabiting coastal lagoons as well as inland salt lakes. Its distribution is not continuous ; the populations are indeed localized in isolated biotopes in both temperate and tropical climates (Stella, 1933). Furthermore it is known that the ecological and physico-chemical characteristics of *Artemia* habitats can differ widely (Cole and Brown, 1967 ; Persoone and Sorgeloos, 1980).

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

[2] "Bevoegdverklaard Navorser" at the Belgian National Foundation for Scientific Research (NFWO).

The geographical isolation of brine shrimp populations has resulted in numerous geographical strains. The number of different *Artemia* populations described today already exceeds 150 (Persoone and Sorgeloos, 1980). Recently, genetic studies of 27 strains revealed the existence of at least six non-interbreeding species (Bowen *et al.*, 1978).

The geographical isolation and the specific habitat conditions have led to various phenotypes with different biological, chemical, and physiological characteristics. Evidence of this has already been presented in a few studies that have been made on these aspects (Gilchrist, 1960 ; Baid, 1963, 1964 ; D'Agostino, 1965 ; Nimura, 1968 ; Lüdskanova and Joshev, 1972 ; Sorgeloos, 1975 ; Claus *et al.*, 1977). Since a more detailed analysis of the various existing *Artemia* populations might lead to a better characterization of their value for specific applications in aquaculture, the "International Study on *Artemia*" was started in 1978 in which the task of the biometrical analysis of brine shrimp strains of different origin was assigned to the Artemia Reference Center.

Materials and methods

Within the framework of the general characterization study of different *Artemia* populations, 17 strains originating from 14 countries have been studied so far. Details on their exact origin are given in Table I. For the strains from San Francisco Bay, Macau, Great Salt Lake, Port Araya and Tuticorin different batches have been analyzed.

It should be noted that the *Artemia* strains from Macau and Barotac Nuevo originate from San Francisco Bay *Artemia* which were inoculated in the two former areas in 1977 and 1978 respectively (Sorgeloos *et al.*, 1979).

The following biometrical parameters have been analyzed : diameter, volume and chorion thickness of the cysts and length, dry weight, ash free dry weight and volume index of the freshly hatched nauplii. The size analysis of the cysts has been performed on both hydrated untreated and decapsulated cysts.

A routine procedure using Coulter Counter® equipment has been worked out in order to measure the processed *Artemia* cysts and to statistically analyse the data obtained (Vanhaecke *et al.*, 1980).

For the analysis of the biometrical characteristics of the nauplii, cysts were incubated in natural seawater (35 ‰) at a temperature of 25 ± 0.5 °C at 1 000 lux. The larvae were usually harvested when 90% of the total number of hatchable nauplii had been produced.

For the slow hatching strains nauplii were sampled 8 to 10 hr after the appearance of the first nauplii. In this way a homogeneous population of instar I nauplii was obtained for all the strains. The nauplii were separated from the hatching debris using a separator box (Persoone and Sorgeloos, 1972). The analyses were made immediately after the separation.

Dry weight analyses were carried out on six replicate series of approximately 50 000 nauplii each ; the number of larvae was checked by taking 10 subsamples of 250 µl. The nauplii were rinced thoroughly with distilled water and dried for 24 hr at 60 °C. After cooling for 30 min in a dessicator the larvae were weighed on a microbalance. The dried samples were then incinerated for 4 hr at 550 °C and the ash weights determined.

For the analysis of the naupliar size, 120 instar I larvae, were fixed in lugol's solution (5 volume %) and the length determined using a microscope equiped with a projection system.

TABLE I

Artemia strains and batches studied

Source of cysts[1]	Batch number or year of harvest	Abbreviation used
Argentina : Buenos Aires (Aquarium Products)	1977	ARG
Australia : – Adelaïde	–	AD
– Shark Bay (World Ocean)	–	SB1
	114	SB2
Brazil : Macau (Cirne Brand)	March 1978	MAC1
	May 1978	MAC2
	October 1978	MAC3
	870191	MAC4
	87500	MAC5
	871172	MAC6
Canada : Chaplin Lake	1978	CHA
China : locality unknown	–	CHI
Colombia : Galera Zamba	1977	GZ
France : Aigues Mortes	–	AM
India : Tuticorin	–	TUT1
	1978	TUT2
Italy : Margherita di Savoia	1977	MS
Philippines : Barotac Nuevo	1978	PHIL
Puerto Rico : Bahia Salinas	–	PR
Spain : San Lucar	1978	SL
USA : – Great Salt Lake	1966	GSL1
	1977	GSL2
– San Francisco Bay (Metaframe-San Francisco Bay Brand, Inc.)	288-2596	SFB1
	288-2606	SFB2
	2847	SFB3
	236-2013	SFB4
	933235	SFB5
– San Pablo Bay (Living World, San Francisco Bay Brand, Inc.)	1628	SPB
Venezuela : Port Araya	August 1977	PA1
	January 1978	PA2
	May 1978	PA3

[1] For those cysts which were purchased from a commercial dealer the company name is given in parenthesis.

The average volume of approximately 20 000 nauplii was measured using Coulter Counter® equipment. The operation conditions on the Coulter Counter® equipment differ from those reported in Vanhaecke *et al.*, (1980) with regard to :

- tube orifice : 1 000 μm
- l/amplification : 4
- count range : 100

In order to slow down the movements of the larvae, which interfere with the proper functioning of the Coulter Counter®, 100 ml glycerol was added to 1 l of the conventional 10 ‰ electrolyte solution. The data obtained do not represent the real volume of the nauplii,

but provide us with a volume index, which is the mean of the volumes of the nauplii which pass through the tube's orifice under different orientations.

Results

The data on the cyst diameter and chorion thickness are summarized in Table II. The volumes of the cysts are represented graphically in Fig. 1. The statistical comparison of the results obtained is given in Table III.

TABLE II

Diameter and chorion thickness of cysts from different *Artemia* strains and batches

Strain[1]	Diameter of hydrated untreated cysts (μm)	s[3]	Diameter of hydrated decapsulated cysts (μm)	s[3]	Chorion thickness (μm)
SFB 1	224.7	12.4	210.0	12.7	7.35
SFB 2	224.6	11.9	210.5	12.3	7.05
SFB 3	223.9	11.7	209.7	12.8	7.10
SFB 4	224.3	11.8	207.7	11.1	8.30
SFB 5	228.7	12.3	212.1	11.3	8.30
SPB	235.6	13.0	220.4	14.3	7.60
PHIL	228.0	13.0	213.8	12.2	7.25
MAC 1	232.5	11.1	216.6	11.4	7.95
MAC 2	227.8	11.7	211.2	12.4	8.30
MAC 3	227.4	11.9	213.2	11.3	7.10
MAC 4	227.7	12.5	212.9	11.3	7.40
MAC 5	231.8	12.3	217.6	12.8	7.10
MAC 6	228.7	11.0	213.8	12.0	7.45
MAC lab[2]	226.9	12.4	211.0	12.5	7.95
GSL 1	252.5	13.0	241.6	13.2	5.45
GSL 2	244.2	16.1	234.8	16.0	4.70
SB 1	259.7	9.7	242.9	10.1	8.40
SB 2	260.4	10.4	242.2	11.3	9.10
PA 1	246.7	12.7	226.5	12.7	10.10
PA 2	246.8	13.4	226.4	14.4	10.20
PA 3	249.0	12.6	226.6	12.8	11.20
TUT 1	283.8	10.2	262.0	11.0	10.90
TUT 2	282.9	14.4	262.7	11.5	10.10
AD	225.8	10.9	209.8	9.5	8.00
ARG	238.2	13.2	217.4	13.9	10.40
PR	253.7	13.3	233.4	13.7	10.15
CHA	240.0	16.1	229.3	15.1	5.35
GZ	249.9	12.3	232.7	11.2	8.60
CHI	267.0	19.8	246.6	18.9	10.20
MS	284.9	14.6	266.3	14.8	9.30
SL	253.6	11.7	237.1	12.2	8.25
AM	259.6	14.1	240.8	14.3	9.40

[1] Legend to the abbreviations in Table I.

[2] MAC lab : cysts produced in a laboratory culture of MAC 2 *Artemia*.

[3] Standard deviation.

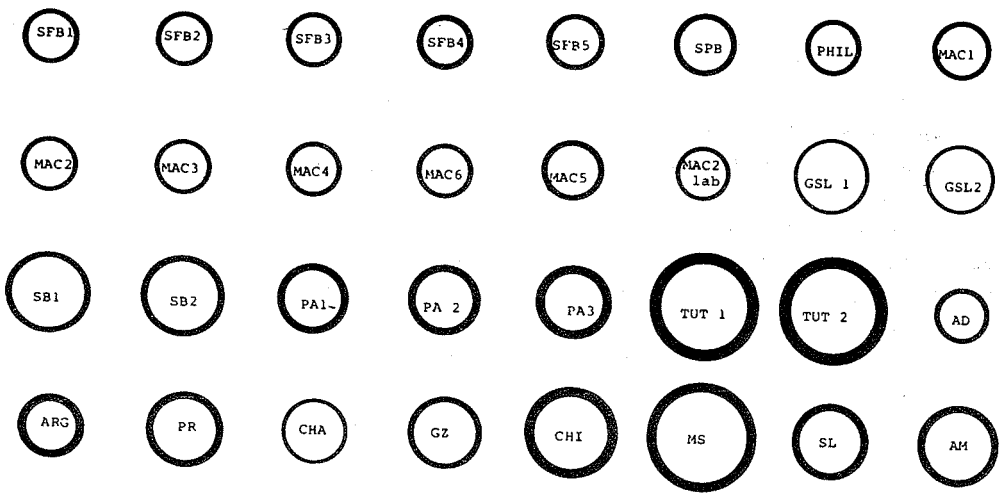

FIG. 1. Schematic diagram of cyst volume (outer circle) and chorion volume (black surface) for different *Artemia* strains and batches. (legend to abbreviations in Table I).

TABLE III

Statistical comparison (0.01 level[1]) of the sizes of *Artemia* cysts from different geographical origin

A. diameter of untreated hydrated cysts
B. diameter of hydrated decapsulated cysts
C. chorion thickness

(Legend to abbreviations in Table I)

A SFB 3 SFB 4 SFB 2 SFB 1 AD MAC 2 lab MAC 3 MAC 4 MAC 2
PHIL SFB 5 MAC 6 MAC 5 MAC 1 SPB ARG CHA GSL 2
PA 1 PA 2 PA 3 GZ GSL 1 SL PR AM
SB 1 SB 2 CHI TUT 2 TUT 1 MS

B SFB 4 SFB 3 AD SFB 1 SFB 2 MAC 2 lab MAC 2 SFB 5
MAC 4 MAC 3 MAC 6 PHIL MAC 1 ARG MAC 5 SPB
PA 2 PA 1 PA 3 CHA GZ PR GSL 2 SL
AM GSL 1 SB 2 SB 1 CHI TUT 1 TUT 2 MS

C GSL 2 CHA GSL 1 SFB 2 SFB 3 MAC 3 MAC 5 PHIL
SFB 1 MAC 4 MAC 6 SPB MAC 1 MAC 2 lab AD SL
SFB 4 SFB 5 MAC 2 SB1 GZ SB 2 MS AM
PA 1 PR PA 2 CHI ARG TUT 2 TUT 1 PA 3

[1] Horizontal lines join cyst sizes that are not significantly different.

From the data it is clear that there are important differences in cyst size and chorion thickness among the different strains.

The mean diameter of the untreated hydrated cysts varies between 220 and 285 μm. The differences observed within strains are very small in comparison to the large variations among the strains. At first we could not understand the 10 μm difference between the San Francisco Bay Brand batch (SPB), labeled San Francisco Bay brine shrimp, and the SFB-batches, until we were informed that the former cyst sample was from the San Pablo Bay in the Nappa Valley, north of San Francisco (Schmidt, personal communication). As such San Pablo Bay *Artemia* should be considered as a separate geographical strain.

The marked difference, which can be observed between the batches of Great Salt Lake, sampled in 1966 and in 1977, might be due to the considerable ecological changes which have occured during the last decades in this salt lake (Stephens and Gillespie, 1976).

It is interesting to note that the cysts harvested in Macau (Brazil) and Barotac Nuevo (Philippines) show only minor differences as compared to the parental SFB-stock. Similarly, the cysts produced by MAC 2 adults in laboratory cultures on a rice bran diet (Versichele and Sorgeloos, 1980) are not significantly different from the original MAC 2 batch.

For the decapsulated cysts, a similar range of differences as in the untreated cysts is observed between the different strains. The cyst diameter varies between 203 and 266 μm. The strain sequence from small to big has, however, changed, indicating differences of chorion thickness. Indeed this parameter varies from 4.7 to 11.2 μm in the batches analyzed.

The results of the size analysis of SFB 1 cyts produced in the laboratory under different salinity conditions (Table IV) reveal only minor differences. Nonetheless the statistical analysis indicates that the cysts produced at 180 ‰ are significantly smaller than the cysts produced at lower salinities.

TABLE IV

The influence of salinity on the biometrics of SFB cysts produced in laboratory cultures

Salinity (‰)	Diameter of hydrated untreated cysts (μm)	s[1]	Diameter of hydrated decapsulated cysts (μm)	s[1]	Chorion thickness (μm)
35	223.5	14.6	206.4	13.7	8.55
90	223.7	16.7	206.6	14.6	8.55
180	222.0	15.3	205.2	13.0	8.40

[1] Standard deviation.

Microscopic measurements of 100 cysts produced at respectively 22 °C and 28 °C from SFB 1 parents revealed no significant size differences between these cysts, the mean size being 223.0 respectively 223.2 μm.

The fact that the laboratory produced cysts are slightly smaller than the original SFB 1 stock is probably due to the presence of empty cyst shells in these samples. Indeed, since these cysts were not dried, the separation (in tapwater) of the empty and broken shells from the full cysts

is less efficient. Empty shells shift the frequency distribution of cysts (as analyzed with Coulter Counter® equipment) to the left.

The data on the dry weight, organic weight and ash content of the nauplii are given in Table V.

TABLE V

Individual dry weight, organic weight and ash content of instar I nauplii of different geographical origin (Legend to abbreviations in Table I)

Strain	Dry weight (μg)	s[1]	Ash content (% of dry weight)	s[1]	Organic weight (μg)	s[1]
SFB 1	1.63	0.11	6.33	0.15	1.52	0.10
SFB 2	1.61	0.09	6.17	0.10	1.51	0.09
SPB	1.92	0.08	5.62	0.15	1.81	0.07
MAC 2	1.68	0.11	5.83	1.11	1.58	0.10
MAC 6	1.74	0.08	5.88	0.03	1.64	0.07
PHIL	1.68	0.03	6.07	0.10	1.58	0.03
GSL 1	2.70	0.13	5.74	0.05	2.55	0.12
GSL 2	2.42	0.11	5.69	0.25	2.28	0.11
SB	2.47	0.13	5.28	0.10	2.34	0.13
PR	2.10	0.11	5.51	0.06	1.99	0.11
GZ	2.27	0.08	6.32	0.07	2.12	0.08
PA 3	2.07	0.09	6.39	0.25	1.94	0.08
ARG	1.72	0.07	6.32	0.28	1.61	0.06
CHI	2.76	0.14	6.25	0.26	2.62	0.14
MS	3.33	0.18	6.17	0.15	3.13	0.17

[1] Standard deviation.

From the dry weight data it appears that there are very large differences between the geographical strains analyzed. The percentual differences among the strains studied, vary from 5 to over 100%. Student's t-tests reveal that the variations within a same strain are not significant, exception made, however, for the already mentioned "San Francisco Bay Brand-1628" batch, which in fact is the San Pablo Bay strain, and the Great Salt Lake 1966 and 1977 batches.

Again no significant differences can be observed between the nauplii from the San Francisco Bay strain and those originating from San Francisco Bay inoculations in either Brazil or the Philippines.

From the data it also appears that the variation in ash content is small, *i.e.* from 5.28 to 6.39% of the naupliar dry weight. As a result we can conclude that the differences in organic weight are proportional to the dry weight differences.

From Fig. 2 it is clear that almost the same range of differences as already given for the dry weights, is valid for the volume index of the nauplii. This parameter varies from 7.6 up to 8.3 for the San Francisco Bay, Macau and Barotac Nuevo nauplii, as compared to 11.4 up to 13.6 for the parthenogenetic strains from China and Italy.

The data for naupliar length are represented graphically in Fig. 3. The maximal difference in naupliar size is about 100 μm.

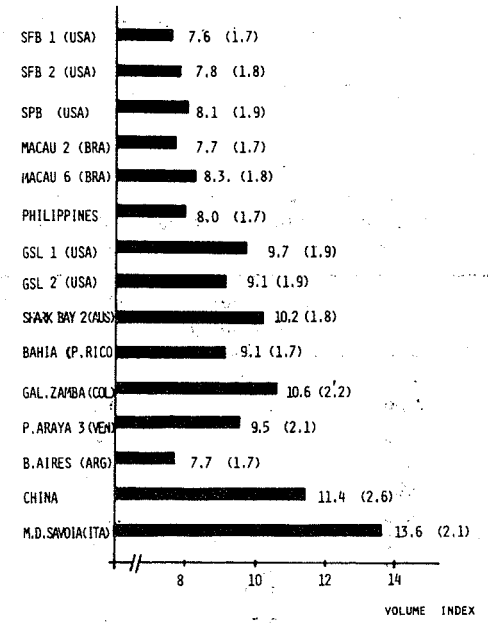

FIG. 2. Volume index of *Artemia* nauplii from different geographical strains (s-values in parentheses ; legend to abbreviations in Table I).

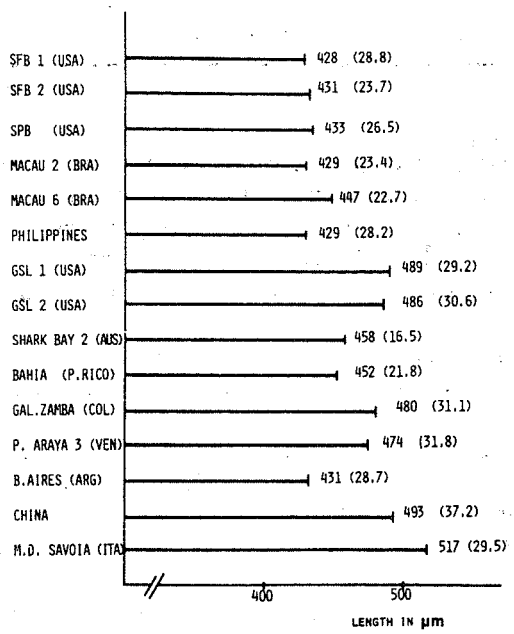

FIG. 3. Length of *Artemia* nauplii from different geographical strains (s-values in parentheses ; legend to abbreviations in Table I).

Discussion

The data obtained so far demonstrate that significant differences exist for several biometrical parameters between *Artemia* strains. Our results confirm the findings of D'Agostino (1965) and Claus *et al.* (1977) with regard to cyst sizes.

The former author reported that within a same strain the mean egg size remains constant between batches which were collected at different periods of the year. Our results, however, indicate that in a few cases the mean cyst size varies significantly among batches from the same strain.

These small variations may be due to changing environmental conditions in the salt ponds. Indeed, it appeared from laboratory tests that a salinity drop from 180 to 90 ‰ causes a ± 1.5 μm increase in the size of both hydrated untreated and decapsulated cysts. Collins (1978) analyzed various *Artemia* strains and did not find a correlation between cyst size and brine density in the natural habitat. Whereas it appears from our studies that temperature, within the experimental range, probably does not influence the cyst size, other factors such as variations in food conditions for the adults or widely separated harvest sites – especially in very large salt ponds – may cause small differences in cyst size among batches.

The packaging technique can eventually also lead to some small differences ; *e.g.* segregation can occur during filling of consecutive cans in function of different cyst densities. Although for most commercial batches analyzed we did not find significant variations, differences ranging from 1.1 up to 1.7 μm were found between cans in specific batches from Macau and San Pablo Bay.

The constancy in cyst size of San Francisco Bay cysts harvested from natural populations and those produced from the same strain but in laboratory conditions confirm similar observations of D'Agostino (1965) with Great Salt Lake *Artemia*.

In conclusion it appears that the biometrical parameters which we studied are mainly strain specific. As a consequence biometrical parameters in general, and more specifically cyst characteristics can be considered as good tools for the characterization of *Artemia* strains. These criteria can from now on be utilized to differentiate strains and to help to define the origin of unspecified cyst samples.

The differences which we have observed for the cyst's characteristics are not correlated with the genetic distances calculated by Abreu-Grobois and Beardmore (1980) for the same geographical strains. More biometrical parameters as well as other criteria (*e.g.* morphological and/or physiological) will probably be needed to evaluate genetic differences between strains.

From our data we distinguish three groups of strains :

1. Those with the smallest cysts produced by the Adelaide-strain and the brine shrimp from the San Francisco Bay area, including the SFB inoculated strains from Macau and Barotac Nuevo ;
2. The parthenogenetic strains from China, France, Italy and India characterized by a large cyst size. This quantitative property might be correlated with the degree of ploidy ;
3. Strains with cysts of intermediate size but with the thinnest chorion characteristic for the *Artemia franciscana* strains described by Bowen and Sterling (1978) from Chaplin Lake and Great Salt Lake.

TABLE VI

Correlations between biometrical characteristics of nauplii and cysts from various *Artemia* strains

Correlation	r-value
Volume of decapsulated cysts – naupliar dry weight	0.986
Volume of untreated cysts – naupliar volume	0.960
Volume of decapsulated cysts – naupliar volume	0.955
Volume of untreated cysts – naupliar dry weight	0.945
Naupliar volume – naupliar dry weight	0.945
(Naupliar length)^3 – naupliar dry weight	0.945
(Naupliar length)^3 – naupliar volume	0.912
Diameter of decapsulated cysts – naupliar length	0.906
Diameter of untreated cysts – naupliar length	0.864

Since one can expect a high correlation between the biometrical characteristics of the cysts and their respective nauplii, a detailed correlation analysis has been carried out for the various parameters studied (Table VI).

Since highly significant correlations were indeed found, the screening of *Artemia* strains can be much simplified with regard to the number of biometrical characteristics to be taken into consideration. For example the correlation between the volume of decapsulated cysts (X) and the naupliar dry weight (Y) is given by the equation $Y = -0.0978 + 3.554.10^{-4}X$. The regression line and its 95% confidence limits are represented graphically in Fig. 4.

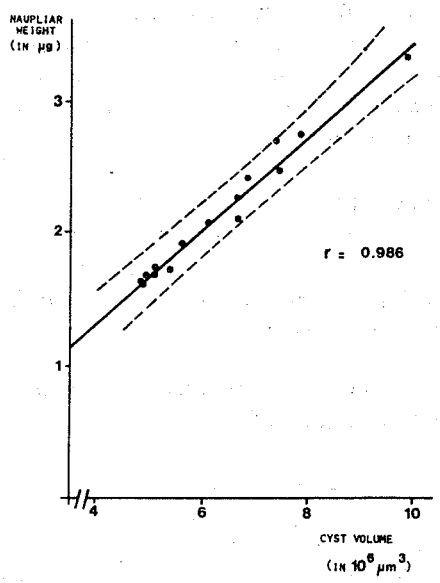

FIG. 4. Regression line and 95% confidence limits for the correlation between the volume of decapsulated cysts and the naupliar dry weight in several *Artemia* strains.

An estimation of the dry weight (with a 95% accuracy, *i.e.* ± 0.22 µg) is now possible by extrapolation from the cyst volume, which can be measured much faster and more precizely using Coulter Counter® equipment.

The highest correlation between the naupliar characteristics is found between the volume index and the dry weight of the nauplii. In fact, for the evaluation of the ingestibility of *Artemia* as a prey, the most realistic criterion to express the amount of food which a nauplius represents is the volume index.

The results obtained so far thus become already of practical value with regard to the selection of the most appropriate strain(s) as an adequate food source for larval fishes or crustaceans in aquaculture hatcheries.

If the size of the *Artemia* prey does not cause ingestion problems for the predator, one might expect that the use of large nauplii with a higher individual organic weight will be beneficial. The predator will indeed spend less energy taking up a smaller number of larger nauplii to fulfill its food demand. This is especially the case for fish larvae which are not very efficient in prey hunting (Rosenthal, 1969). The beneficial effect of feeding bigger *Artemia* is apparent from the experimental results of Beck *et al.* (1980) : *Menidia* larvae indeed grew significantly faster on a diet of large nauplii from Margherita di Savoia, Great Salt Lake and Shark Bay as compared to those silverside larvae fed with the smaller nauplii from San Francisco Bay and Macau.

In a similar comparative study with *Pseudopleuronectes americanus* larvae Klein-MacPhee *et al.* (1980) also noted better growth results for larval winter flounder raised on Shark Bay and Margherita di Savoia nauplii as compared to Macau, San Pablo Bay and Great Salt Lake nauplii. As reported by the same authors the poor growth results obtained with the SPB and especially with the relatively large GSL larvae are not related to prey size but appear to be caused by nutritional and/or toxic factors.

For those cultured organisms where the naupliar size is critical for the ingestion mechanism of the predator, better growh might be expected when using small nauplii. The use of a particular *Artemia* strain may even result in a total failure in culturing a specific predator on brine shrimp because of the inability of the predator to ingest this specific *Artemia* strain (Smith, 1976). As a consequence the larval age of the predator at which a diet of freshly hatched *Artemia* nauplii can be successfully used is function of the strain of brine shrimp used ; *e.g.* Beck *et al.* (1980) compared the biological effectiveness of freshly hatched nauplii from MAC, SPB, SB, GSL and MS in feeding trials with newly hatched *Menidia* larvae ; in the group of fish which were offered large MS nauplii (volume index 13.6) a high mortality, similar to the one noted for the starved fish, was recorded during the first 3 days of the experiment ; after this time the mortality of the MS fed fish larvae did not exceed the mortality of those fed smaller nauplii from the other strains. A selection of specific *Artemia* strains based on biometrical characteristics might thus be very useful to raise the chance of success in rearing specific organisms.

From the foregoing it becomes clear that the comparative study on *Artemia* strains should be continued by screening more strains, taking advantage however, of the earlier mentioned correlations, which facilitate the characterization study. We intend to also study other correlations such as : cyst size versus adult size ; chorion thickness and light intensity threshold at the onset of the hatching metabolism ; naupliar size versus larval growth rate and finally biometrical characteristics in function of the various genotypes.

At a later stage cross-breeding of specific strains with particular characteristics will be considered and the heritability of these parameters in the new strains produced will be studied.

Acknowledgements

We are very indebted to the many people who provided us with cyst samples from various geographical strains of brine shrimp.

This research has been supported by the Belgian National Foundation for Scientific Research (NFWO) through Grant FKFO, No. 2.0010.78.

Literature cited

ABREU-GROBOIS F. A., J. A. BEARDMORE. 1980. International Study on *Artemia*. II. Genetic characterization of *Artemia* populations. p. 133-146. *In* : The brine shrimp *Artemia*. Vol. 1. Morphology, Genetics, Radiobiology, Toxicology. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 345 p.

BAID I. C. 1963. The effect of salinity on growth and form of *Artemia salina* (L.). *J. exp. Zool.* 153(3):279-284.

BAID I. C. 1964. Neoteny in the genus *Artemia*. *Acta Zool.* 45:167-177.

BECK A. D., D. A. BENGTSON, and W. H. HOWELL. 1980. International Study on *Artemia*. V. Nutritional value of five geographical strains of *Artemia* : effects on survival and growth of larval Atlantic silverside *Menidia menidia*. p. 249-259. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

BOWEN S. T., J. P. DURKIN, G. STERLING, and L. S. CLARK. 1978. *Artemia* hemoglobins : genetic variation in parthenogenetic and zygogenetic populations. *Biol. Bull.* 155:273-287.

BOWEN S. T. and G. STERLING. 1978. Esterase and malate dehydrogenase isozyme polymorphisms in 15 *Artemia* populations. *Comp. Biochem. Physiol.* 61B:593-595.

CLAUS C., F. BENIJTS, and P. SORGELOOS. 1977. Comparative study of different geographical strains of the brine shrimp *Artemia salina*. p. 91-105. *In* : Fundamental and applied research on the brine shrimp, *Artemia salina* (L.) in Belgium. Jaspers E. and G. Persoone (Eds). European Mariculture Society Special Publication No. 2. EMS, Bredene, Belgium. 110 p.

COLE G. A. and R. J. BROWN. 1967. Chemistry of *Artemia* habitats. *Ecology* 48(5):858-861.

COLLINS N. C. 1978. The ecological significance of egg size differences among brine shrimp (*Artemia salina*) populations. *Bull. Ecol. Soc. America* 59(2):57.

D'AGOSTINO A. S. 1965. Comparative studies of *Artemia salina* (development and physiology). Thesis, New York University. 83 p. University Microfilms, 66-5653.

GILCHRIST B. M. 1960. Growth and form of the brine shrimp, *Artemia salina* (L.). *Proc. zool. Soc. Lond.* 134(2): 221-235.

KLEIN-MACPHEE G., A. D. BECK, and W. H. HOWELL. 1980. International Study on *Artemia*. VII. Nutritional value of five geographical strains of *Artemia* : effects on survival and growth of larval winter flounder *Pseudopleuronectes americanus*. p. 305-312. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

LÜDSKANOVA J. and L. JOSHEV. 1972. Die Anzucht von *Artemia salina* L. als Nahrung für Pflanzenfresser. *Z. Binnenfischerei DDR* 19(6):177-181.

NIMURA Y. 1968. Note on hatching the cyst of *Artemia*. *Aquiculture* 16(2):105-115. (in Japanese).

PERSOONE G. and P. SORGELOOS. 1972. An improved separator box for *Artemia* nauplii and other phototactic invertebrates. *Helgoländer wiss. Meeresunters.* 23:243-247.

PERSOONE G. and P. SORGELOOS. 1980. General aspects of the ecology and biogeography of *Artemia*. p. 3-24. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

ROSENTHAL H. 1969. Verdauungsgeschwindigkeit, Nahrungswahl und Nahrungsbedarf bei den Larven des Herings, *Clupea harengus* L. *Ber. Dtsch. wiss. Komm. Meeresforsch.* 20:60-69.

SMITH W. E. 1976. Larval feeding and rapid maturation of bluegills in the laboratory. *Progve Fish-Cult.* 38(2):95-97.

SORGELOOS P. 1975. De invloed van abiotische en biotische faktoren op de levenscyclus van het pekelkreeftje, *Artemia salina* L. Ph. D. Thesis, State University of Ghent. 235 p.

SORGELOOS P., E. BOSSUYT, E. BRUGGEMAN, A. COOREMAN, J. DOBBELEIR, P. VANHAECKE, and D. VERSICHELE. 1979. Progress report on the research activities of the *Artemia* Reference Center. *ICES C.M./F:54.* 206-214.

STELLA E. 1933. Phenotypical characteristics and geographical distribution of several biotypes of *Artemia salina* L. *Z. ind. Abst. Vererbungslehre* 65:412-446.

STEPHENS D. W. and D. M. GILLESPIE. 1976. Phytoplankton production in the Great Salt Lake Utah USA, and a laboratory study of algal response to enrichment. *Limnol. Oceanogr.* 21(1):74-87.

VANHAECKE P., H. STEYAERT, and P. SORGELOOS. 1980. International Study on *Artemia*. III. The use of Coulter Counter® equipment for the biometrical analysis of *Artemia* cysts – methodology and mathematics. p. 107-115. *In* : The brine shrimp *Artemia*. Vol. 1. Morphology, Genetics, Radiobiology, Toxicology. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 345 p.

VERSICHELE D. and P. SORGELOOS. 1980. Controlled production of *Artemia* cysts at laboratory scale. p. 231-246. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.